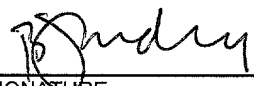| FORM PTO-1390 (REV 11-2000)	U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 620-136

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U S APPLICATION NO (If known, see 37 C F R. 1 5)

09/806326
Unknown

INTERNATIONAL APPLICATION NO. PCT/GB99/03264	INTERNATIONAL FILING DATE 4 October 1999	PRIORITY DATE CLAIMED 2 October 1998 2 October 1998 15 February 1999

TITLE OF INVENTION

SUSCEPTIBILITY LOCUS FOR OSTEOARTHRITIS

APPLICANT(S) FOR DO/EO/US

SYKES et al. Bryan ·

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f). The submission must include items (5), (6), (9) and (21) indicated below.

4. ☒ The U.S. has been elected by the expiration of 19 months from the priority date (Article 31).

5. ☐ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).

   a. ☐ is attached hereto (required only if not communicated by the International Bureau).

   b. ☒ has been communicated by the International Bureau.

   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).

   a. ☐ is attached hereto.

   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))

   a. ☐ are attached hereto (required only if not communicated by the International Bureau).

   b. ☐ have been communicated by the International Bureau.

   c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.

   d. ☐ have not been made and will not be made.

8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

   Items 11 To 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.

14. ☐ A SECOND or SUBSEQUENT preliminary amendment.

15. ☐ A substitute specification.

16. ☐ A change of power of attorney and/or address letter.

17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.

18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).

19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).

20. ☒ Other items or information. PTO-1449 and International Search Report

U.S. APPLICATION NO (If known, see 37 C.F.R. 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
Unknown 09/786326	PCT/GB99/03264	620-136

21. ☒ The following fees are submitted: | **CALCULATIONS** PTO USE ONLY

BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):

-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1000.00

-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO....................$860.00

-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO.......................$710.00

-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)...............................$690.00

-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4).....................................$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT = $ 860.00

Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)). $ 130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE			
Total Claims	22	-20 =	2	X	$18.00	$ 36.00
Independent Claims	16	-3 =	13	X	$80.00	1040.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			$270.00			$ 0.00

TOTAL OF ABOVE CALCULATIONS = $ 2066.00

☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. 0.00

SUBTOTAL = $ 2066.00

Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)). + 0.00

TOTAL NATIONAL FEE = $ 2066.00

Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). **$40.00** per property + $ 0.00

Fee for Petition to Revive Unintentionally Abandoned Application ($1240.00 – Small Entity = $620.00) $ 0.00

TOTAL FEES ENCLOSED = $ 2066.00

| Amount to be: refunded | $ | |
| Charged | $ | |

a. ☒ A check in the amount of $2066.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.
d. ☒ The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

B. J. Sadoff
NAME

36,663   **March 29, 2001**
REGISTRATION NUMBER   Date

- 2 -

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

SYKES et al.   Atty. Ref.:   **620-136**

Serial No.   **Unknown**   Group:

National Phase of: **PCT/GB99/03264**
International Filing Date: **4 October 1999**

Filed: **March 29, 2001**   Examiner:

For: **SUSCEPTIBILITY LOCUS FOR OSTEOARTHRITIS**

*   *   *   *   *   *   *   *   *   *   *

March 29, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to calculation of the filing fee and in order to place the above identified

application in better condition for examination, please amend the claims as follows:

IN THE CLAIMS

Please substitute the following amended claims for corresponding claims

previously presented. A copy of the amended claims showing current revisions is

attached.

12. (Amended) A method for isolating genetic loci according to claim 7 in which

open reading frames are identified within 500 Kb of said allele.

19. (Amended) A method for determining individual susceptibility to osteoarthritis

according to claim 13 in which one or more of the following genomic regions is

additionally analysed; a genomic region comprising the polymorphic marker D6S273

and a genomic region comprising the polymorphic marker DXS1068.

REMARKS

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

The above amendments are made to place the claims in a more traditional format.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

12. (Amended) A method for isolating genetic loci according to [any one of claims 7 to 11] claim 7 in which open reading frames are identified within 500 Kb of said allele.

19. (Amended) A method for determining individual susceptibility to osteoarthritis according to [any one of claims 13 to 15] claim 13 in which one or more of the following genomic regions is additionally analysed; a genomic region comprising the polymorphic marker D6S273 and a genomic region comprising the polymorphic marker DXS1068.

SUSCEPTIBILITY LOCUS FOR OSTEOARTHRITIS

Technical Field

This invention relates to the identification of chromosomal
5   regions linked to susceptibility to osteoarthritis using
linkage and association analysis.

Background of Invention

Osteoarthritis (OA) is a debilitating disease involving
10   degeneration of the articular cartilage of synovial joints[5,6].
Although OA has long been considered an inevitable
consequence of ageing, it has become increasingly apparent
that OA does have a genetic component. Early-onset forms of
the disease are associated with several
15   osteochondrodysplasias, rare diseases involving abnormal bone
and cartilage development that are transmitted as Mendelian
traits[7]. However, the OA in these conditions is secondary to
the main dysplastic phenotype. The common late-onset form of
the disease (idiopathic OA) often has no obvious
20   environmental (i.e. injury) or characteristic physical cause
and does not demonstrate a clear mode of inheritance.

Over the last 10 years, many genes for single gene or
monogenic diseases, which are relatively rare in the
25   population, have been positioned by linkage analysis in
families, and localised to a small enough region to allow
identification of the gene. The latter sublocalisation and
fine mapping can be carried out in single gene rare diseases
because recombinations within families define the boundaries
30   of the minimal interval beyond any doubt. In contrast, in
common diseases such as osteoarthritis, diabetes or asthma
the presence of the disease mutation does not always coincide
with the development of the disease: disease susceptibility
mutations in common disorders provide risk of developing of
35   the disease, and this risk is usually much less than 100%.

2

Hence, susceptibility genes in common diseases cannot be
localised using recombination events within families, unless
tens of thousands of families are available to fine map the
locus. Because collections of this size are impractical,
5   investigators are contemplating the use of association
mapping, which relies on historical recombination events
during the history of the population from which the families
came from.

10   Association mapping has been used in over a dozen examples of
rare single gene traits, and particularly in genetically
isolated populations such as Finland to fine map disease
mutations. Nevertheless, association mapping is
fundamentally different from straightforward linkage mapping
15   because even though the degree of association between two
markers or a marker and a disease mutation is proportional to
the physical distance along the chromosome this relationship
can be unpredictable because it is dependent on the allele
frequencies of the markers, the history of the population and
20   the age and number of mutations at the disease locus. For
rare, highly penetrant single gene diseases there is usually
one major founder chromosome in the population under study,
making it relatively feasible to locate an interval that is
smaller than one that can be defined by standard
25   recombination events within living families. The resolution
of this method in monogenic diseases in which there is one
main founder chromosome is certainly less than 2cM, and in
certain examples the resolution is down to 100 kb of DNA
(Hastbacka et al. (1994) Cell 78,1-20).

30

In common diseases like OA, which are caused by a number of
genes or polygenes acting together in concert the population
frequency of the disease allele may be very high, perhaps
exceeding 50%, and there are likely to be several founder
35   chromosomes, all of which impart risk, and not a 100%

certainty of disease development. Because association
mapping is dependent on unpredictable parameters, and
because founder chromosomes will be several and common in
frequency in the general population, the task of fine mapping
polygenes is currently one of some controversy, and many
doubt the feasibility at all of a systematic genetic approach
using a combination of linkage and association mapping.
Recently, Risch and Marakandis have provided some
mathematical background to the feasibility of association
mapping in complex diseases (*Science* 273 1516-1517, 1996) but
they did not take into account the effect of multiple founder
chromosomes.

It has often been noted in epidemiological studies that there
is a female preponderance for OA[5,6]. This may be accounted
for by differential effects on the two sexes of environmental
factors. However, a Finnish twin study has suggested that
genetic susceptibility may be greater in women than men[9].
This result has recently been supported by a segregation
analysis[3]. Not only have differences in risk between females
and males been reported but it has also been suggested that
there are differences in heritability between joint
groups[2,10,11]. These differences could be the result of genetic
locus heterogeneity between the different joints.

Summary of the Invention

The present inventors have identified regions on chromosome
2q that may harbour susceptibility loci for OA. This region
was identified following a two-stage non-parametric linkage
analysis. The first stage involved a random screen of the
genome using 272 microsatellite markers in 297 OA families.
The second stage was more selective and involved genotyping
an additional 184 families for those markers that
demonstrated moderate evidence of linkage in stage 1. This
revealed one microsatellite on chromosome 2 for which the

4

evidence for linkage increased as the number of families studied increased. Finer mapping in and around this microsatellite was performed which provided enhanced evidence for linkage and enabled linked regions to be defined.

5   Stratification analysis suggested that the chromosome 2 loci may have differential penetrance between males and females and between the two different joint groups examined (hips and knees).

10   Linkage on chromosome 2 may encompass three distinct loci: a locus close to D2S114, a locus in-between D2S2330 and D2S326 and a locus close to or in-between D2S117 and D2S325. None of the single or multipoint LOD scores for these three regions are particularly high, with the highest single point

15   LOD score being 2.36 at marker D2S202 and the highest MMLS (Maximum multipoint log score) being 2.07 in-between markers D2S117 and D2S72, both in hip-only pairs. What is more striking about our linkage results for chromosome 2 is that there is overlap with regions that other groups have

20   previously identified as potentially harbouring OA susceptibility loci[12,13]. This provides corroboratory evidence that the linkages to chromosome 2 that we have detected are real.

25   Some evidence has also been obtained for linkage with the polymorphic chromosome 6 marker D6S273 and the polymorphic X chromosome marker DXS1068. This may indicate the presence of loci on these chromosomes that also have a role in OA susceptibility. These loci may interact with the loci

30   identified on chromosome 2 and play a role in the differential penetration observed between males and females.

Open reading frames (ORFs) are stretches of genetic sequence which are candidates for being expressed genes. They can be

35   identified by the presence of sequence elements which are

characteristic of coding sequence, such as sequence elements
from exon-intron boundaries, transcriptional initiation and
termination motifs and start and stop codons. Because large
amounts of sequence can be screened rapidly for these
5   elements, the identification of ORFs is commonly an initial
step in the discovery of novel genes.

Microsatellite marker loci are designated in this application
according to the nomenclature conventional in the field of
10   Human Genetics. This provides a unique designation which
specifically and unambiguously identifies each marker locus.
Mapping data for any particular marker locus is readily
available from conventional sources, such as the Gopher
server at the Human Genome Mapping Project Resource Centre
15   (Host = gopher.hgmp.mrc.ac.uk; Port = 70 or URL:
gopher://gopher.hgmp.mrc.ac.us:70/ or by anonymous ftp from
ftp.hgmp.mrc.uk:/Oxford_Primers).

The present invention relates to chromosomal regions linked
20   to genetic sequences which affect susceptibilty to
osteoarthritis.

A   first aspect of the present invention is a method for
identifying individuals susceptible to osteoarthritis
25   comprising obtaining a sample of genomic DNA and detecting
the presence or absence of any one of the 190bp and 200bp
alleles of D2S325 from chromosome 2.

Another aspect of the present invention is a method for
30   identifying male individuals susceptible to osteoarthritis
comprising obtaining a sample of genomic DNA and detecting
the presence or absence of any one of the 190bp and 200bp
alleles of D2S325 from chromosome 2.

Another aspect of the present invention is a method for
identifying male individuals susceptible to osteoarthritis
comprising obtaining a sample of genomic DNA and detecting
the presence or absence of the 208bp allele of D2S117 from

5   chromosome 2.

A further aspect of the present invention is a method for
identifying female individuals susceptible to osteoarthritis
comprising obtaining a sample of genomic DNA and detecting

10   the presence or absence of any one of the 192bp, 202bp and
208bp alleles of D2S117 from chromosome 2.

A further aspect of the present invention is a method for
identifying male individuals susceptible to osteoarthritis of
15   the hip comprising obtaining a sample of genomic DNA and
detecting the presence or absence of the 208 bp allele of
D2S117 from chromosome 2.

Another aspect of the present invention is a method for
20   isolating genetic loci associated with susceptability to OA
comprising screening genomic libraries with sequence from any
one of the 190bp and 200bp alleles of D2S325 and identifying
open reading frames in regions adjacent to said allele.

25   Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA
comprising screening a genomic library from an individual who
is homozygote for any one of the 190bp and 200bp alleles of
D2S325 and identifying open reading frames in regions
30   adjacent to said allele.

Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA
comprising screening a genomic library from an individual who
35   is homozygote for any one of the 190bp and the 200bp alleles

7

of D2S325 and identifying open reading frames located within
500 Kb of D2S325, or more preferably within 100 Kb of D2S325,
or even more preferably within 50 Kb of D2S325 or most
preferably within 10 Kb of D2S325.

Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA
comprising identifying open reading frames in regions
adjacent to D2S325 and comparing said open reading frames in
individuals carrying any one of the 190 bp and 200bp alleles
of D2S325 with said open reading frames in individuals with
other alleles of D2S325.

Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA
comprising identifying open reading frames located within 500
Kb of D2S325, or more preferably within 100 Kb of D2S325, or
even more preferably within 50 Kb of D2S325 or most
preferably within 10 Kb of D2S325 and comparing said open
reading frames in individuals carrying any one of the 190 bp
and 200 bp alleles of D2S325 with said open reading frames in
individuals with other alleles of D2S325.

Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA
comprising screening genomic libraries with sequence from the
192bp and 202bp alleles of D2S117 and identifying open
reading frames in regions adjacent to said allele.

Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA
comprising screening a genomic library from an individual who
is homozygote for any one of the 192bp, the 202bp and the
208bp alleles of D2S117 and identifying open reading frames
in regions adjacent to said allele

8

Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA
comprising screening a genomic library from an individual who
is homozygote for any one of the 192bp, 202bp and 208bp

5   alleles of D2S117 and identifying open reading frames located
within 500 Kb of D2S117, or more preferably within 100 Kb of
D2S117, or even more preferably within 50 Kb of D2S117 or
most preferably within 10 Kb of D2S117.

10   Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA
comprising identifying open reading frames in regions
adjacent to D2S117 and comparing said open reading frames in
individuals carrying any one of the 192bp, 202bp and 208bp

15   alleles of D2S117 with said open reading frames in
individuals with other alleles of D2S117.

Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA
comprising identifying open reading frames located within 500

20   Kb of D2S117, or more preferably within 100 Kb of D2S117, or
even more preferably within 50 Kb of D2S117 or most
preferably within 10 Kb of D2S117 and comparing said open
reading frames in individuals carrying any one of the 192bp,

25   202bp and 208bp alleles of D2S117 with said open reading
frames in individuals with other alleles of D2S117.

Another aspect of the present invention is a method for
isolating genetic loci associated with susceptability to OA

30   comprising screening genomic libraries with sequence from any
one of the 190bp and 200bp alleles of D2S325 and identifying
open reading frames located within 500 Kb of D2S325, or more
preferably within 100 Kb of D2S325, or even more preferably
within 50 Kb of D2S325 or most preferably within 10 Kb of

35   D2S325.

9

Another aspect of the present invention is a method for isolating genetic loci associated with susceptability to OA comprising screening genomic libraries with sequence from any one of the 192bp, 202bp and 208bp alleles of D2S117 and

5   identifying open reading frames located within 500 Kb of D2S117, or more preferably within 100 Kb of D2S117, or even more preferably within 50 Kb of D2S117 or most preferably within 10 Kb of D2S117.

10   Another aspect of the present invention is the use of the 190bp and 200bp alleles of D2S325 as markers for the identification of loci influencing susceptibility to OA.

Another aspect of the present invention is the use of any one

15   of the 192bp, 202bp and 208bp alleles of D2S117 as a marker for the identification of loci influencing susceptibility to OA.

Another aspect of the present invention is a method for

20   mapping loci which affect susceptability to OA by comparing genomic regions containing the 208 bp allele of D2S117 with genomic regions containing other alleles of D2S117.

Another aspect of the present invention is a method for

25   determining individual susceptibility to osteoarthritis comprising obtaining sample genomic DNA from siblings, at least two of which have clinical symptoms of osteoarthritis. analysing a region of their genomic DNA comprising a polymorphic marker, said region being located on chromosome

30   2q between D2S117 and D2S325, identifying allele sharing between the siblings as defined by a maximum log of the odds (LOD) score of greater than 1 and a p-value of less then 0.25, and determining individual susceptibility to osteoarthritis by reference to the allele sharing.

35

10

Another aspect of the present invention is a method for
determining individual susceptibility to osteoarthritis
comprising obtaining sample genomic DNA from siblings, at
least two of which have clinical symptoms of osteoarthritis.
5   analysing a region of their genomic DNA comprising a
polymorphic marker, said region being located on chromosome
2q between D2S202 and D2S72, identifying allele sharing
between the siblings as defined by a maximum log of the odds
(LOD) score of greater than 1 and a p-value of less then
10   0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

Another aspect of the present invention is a method for
determining individual susceptibility to osteoarthritis
comprising obtaining sample genomic DNA from siblings, at
15   least two of which have clinical symptoms of osteoarthritis
analysing a region of their genomic DNA comprising a
polymorphic marker, said region being located on chromosome
2q between D2S117 and D2S325, and additionally analysing one
20   or more of the following; a genomic region comprising the
polymorphic marker D6S273 and a genomic region comprising the
polymorphic marker DXS1068, identifying allele sharing
between the siblings as defined by a maximum log of the odds
(LOD) score of greater than 1 and a p-value of less then
25   0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

Another aspect of the present invention is a method for
determining individual susceptibility to osteoarthritis
30   comprising obtaining sample genomic DNA from siblings, at
least two of which have clinical symptoms of osteoarthritis
analysing a region of their genomic DNA comprising a
polymorphic marker, said region being located on chromosome
2q between D2S202 and D2S72, and additionally analysing one
35   or more of the following; a genomic region comprising the

11

polymorphic marker D6S273 and a genomic region comprising the
polymorphic marker DXS1068, identifying allele sharing
between the siblings as defined by a maximum log of the odds
(LOD) score of greater than 1 and a p-value of less then
0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

Another aspect of the present invention is a method for
determining individual susceptibility to osteoarthritis
comprising obtaining sample genomic DNA from siblings, at
least two of which have clinical symptoms of osteoarthritis.
analysing a region of their genomic DNA comprising the
polymorphic marker D2S114, identifying allele sharing between
the siblings as defined by a maximum log of the odds (LOD)
score of greater than 1 and a p-value of less then 0.25, and
determining individual susceptibility to osteoarthritis by
reference to the allele sharing.

Another aspect of the present invention is a method for
determining individual susceptibility to osteoarthritis
comprising obtaining sample genomic DNA from siblings, at
least two of which have clinical symptoms of osteoarthritis.
analysing a region of their genomic DNA comprising the
polymorphic marker D2S114, and additionally analysing one or
more of the following; a genomic region comprising the
polymorphic marker D6S273 and a genomic region comprising the
polymorphic marker DXS1068, identifying allele sharing
between the siblings as defined by a maximum log of the odds
(LOD) score of greater than 1 and a p-value of less then
0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

Another aspect of the present invention is a method for
determining individual susceptibility to osteoarthritis
comprising obtaining sample genomic DNA from siblings, at

12

least two of which have clinical symptoms of osteoarthritis.
analysing a region of their genomic DNA comprising a
polymorphic marker, said region being located on chromosome
2q between D2S330 and D2S326, identifying allele sharing

5   between the siblings as defined by a maximum log of the odds
(LOD) score of greater than 1 and a p-value of less then
0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

10   Another aspect of the present invention is a method for
determining individual susceptibility to osteoarthritis
comprising obtaining sample genomic DNA from siblings, at
least two of which have clinical symptoms of osteoarthritis.
analysing a region of their genomic DNA comprising a

15   polymorphic marker, said region being located on chromosome
2q between D2S330 and D2S326,and additionally analysing one
or more of the following; a genomic region comprising the
polymorphic marker D6S273 and a genomic region comprising the
polymorphic marker DXS1068, identifying allele sharing

20   between the siblings as defined by a maximum log of the odds
(LOD) score of greater than 1 and a p-value of less then
0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

25   Another aspect of the present invention is a method for
determining individual susceptibility to osteoarthritis
comprising obtaining sample genomic DNA from siblings, at
least two of which have clinical symptoms of osteoarthritis.
analysing a region of their genomic DNA comprising any one of

30   the polymorphic markers; D2S202, D2S72, D3S1266, D4S231,
D4S415, D6S260, D6S273, D6S286, D6S281, D7S669, D7S530,
D11S907, D11S903, D11S901, D17S807, D17S789, DXS1068,
identifying allele sharing between the siblings as defined by
a maximum log of the odds (LOD) score of greater than 1 and a

35   p-value of less then 0.25, and determining individual

13

susceptibility to osteoarthritis by reference to the allele
sharing.

Another aspect of the present invention is a method for
5   determining individual susceptibility to osteoarthritis
comprising obtaining sample genomic DNA from siblings, at
least two of which have clinical symptoms of osteoarthritis.
analysing a region of their genomic DNA comprising any one of
the polymorphic markers D6S273, DXS1068, identifying allele
10   sharing between the siblings as defined by a maximum log of
the odds (LOD) score of greater than 1 and a p-value of less
then 0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

Another aspect of the present invention is a method for
identifying loci conferring susceptibilty to osteoarthritis
comprising screening a genomic library with genetic sequence
derived from one or more of the following polymorphic
markers; D2S202, D3S1266, D4S231, D4S415, D6S260, D6S273,
20   D6S286, D6S281, D7S669, D7S530, D11S907, D11S903, D11S901,
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clones which additionally contain open reading frames

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located within 500 Kb, more preferably within 100 Kb, even
more preferably within 50 Kb or most preferably within 10 Kb
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14

identifying loci conferring susceptibilty to osteoarthritis
comprising screening a genomic library with genetic sequence
derived from one or more of the polymorphic markers D6S273,
DXS1068 and identifying open reading frames located within
500 Kb, more preferably within 100 Kb, even more preferably
within 50 Kb or most preferably within 10 Kb of either of
these polymorphic markers.

Description of Drawings

Figure 1 shows a multipoint log of the odds (LOD) analysis on
the unstratified data.
Figure 2 shows a multipoint log of the odds (LOD) analysis on
stratified data from male sibling pairs only.
Figure 3 shows a multipoint log of the odds (LOD) analysis on
stratified data from hip pairs only.
Figure 4 shows a multipoint log of the odds (LOD) analysis on
stratified data from male/hip pairs only.

Detailed Description of Invention

Linkage Analysis

The present inventors collected 481 families in which at
least two siblings have undergone joint replacement surgery
of the hip or knee for severe, end-stage idiopathic OA (Table
1). Due to the late onset of the disease, none of these
families contained parents who could participate in the
study. In stage 1, 272 microsatellite markers were genotyped
in 297 of the 481 families. The microsatellites were
essentially those used by Reed et al[8] with the replacement of
certain markers that amplified unreliably. Sixteen markers
from stage 1 had evidence of linkage at $p \le 0.05$ (Table 2).
These markers were then genotyped in the remaining 184
families. None of the 16 markers had a $p \le 0.05$ in this second
stage although three had a $p \le 0.10$: D2S202 (p=0.07), D11S903
(p=0.07) and D11S901 (p=0.10) (Table 2). When the data for
stages 1 and 2 were combined and compared to stage 1 only,

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the combined p-value decreased for 4 of the 16 markers:
D2S202 (p=0.009 for combined vs. 0.036 for stage 1), D11S907
(p=0.019 for combined vs. 0.05 for stage 1), D11S903 (p=0.004
for combined vs. 0.017 for stage 1) and D11S901 (p=0.0003 for
5   combined vs. 0.0004 for stage 1) increasing the number of
families had therefore increased the evidence for linkage at
these 4 markers. The present invention is related to the
chromosome 2 marker (D2S202) thus identified.

10   Although D2S202 was the only marker on chromosome 2 that had
a p≤0.05 in stage 1, one other chromosome 2 marker had a
p≤0.10: D2S72 (p=0.07). This marker is approximately 2cM
from D2S202. When D2S72 was genotyped in stage 2 there was
further evidence for linkage with a p-value of 0.024. When
15   screens 1 and 2 were combined a p-value of 0.007 (LOD=1.26)
was obtained for D2S72. This was a lower p-value than for
D2S202 (0.007 vs. 0.009). The factor that two adjacent
markers gave evidence of linkage at p<0.01 prompted us to
genotype the stage 2 families for 6 other chromosome 2
20   markers from our original marker set that flanked D2S202 and
D2S72. In addition, we genotyped 5 new markers for all 481
families. Table 3 lists the results for all 13 chromosome 2
markers that were genotyped in all 481 families. A
multipoint analysis of this data gave a MMLS of 1.09 in-
25   between markers D2S202 and D2S325 (Figure 1). Intriguingly,
the multipoint analysis appears to highlight two additional
distinct linkage regions, one located close to D2S114
(MMLS=0.95) and one located in-between D2S2330 and D2S326
(MMLS=0.91). Overall, these three peaks cover a distance
30   greater than 60cM and therefore probably do not represent a
single locus.

The results were stratified into six categories: those
families that were affected female-only pairs (196 families),
35   affected male-only pairs (male and/or female) (54 families),

16

affected female-only pairs who had undergone hip replacement
but not knee replacement (female/hip pairs) (132 families)
and affected male-only pairs who had undergone hip
replacement but not knee replacement (male/hip pairs) (72

5   families) (Table 1). We did not stratify for female/knee
pairs or male/knee pairs as the number of families were too
low (21 and 8 respectively) and thus any significant results
may simply be the result of stochastic variation.

10   Stratification of chromosome 2 revealed that the
detected linkage was apparently accounted for by affected
male-only pairs, with a single point LOD score of 1.79
($p=0.0021$) at D2S202 and 1.70 ($p=0.0025$) at D2S72 (Table 4).
The MMLS for male-only pairs was 1.02 in-between markers

15   D2S117 and D2S72 (Figure 2). For affected female-only pairs
there was no evidence of linkage at D2S202 ($p=0.40$), D2S72
($p=0.22$) or any of the chromosome 2 markers except for marker
D2S326 ($p=0.019$, 20cM proximal to D2S202). The linkage to
chromosome 2 was restricted to pairs with hip-only disease

20   with a p-value of 0.005 at D2S202 in hip-only pairs versus
0.34 in knee-only pairs. Again, however, the absence of
linkage in knee-only pairs could be due to their relatively
low number. The MMLS in hip-only pairs was 2.07, also in-
between markers D2S117 and D2S72 (Figure 3). This linkage in

25   hip-only pairs was restricted to males, with a p-value at
D2S202 of 0.0016 in male/hips versus 0.20 in female/hips.
The MMLS in male/hips was 1.54, also in-between markers
D2S117 and D2S72 (Figure 4). Overall, these results suggest
that the major linkage to chromosome 2 in our families is

30   restricted to males with OA.
What is very striking about our multipoint analysis of
chromosome 2 is that in the un-stratified and the stratified
analysis there was at least one additional peak besides the
major peak (Figures 1 to 4). These peaks may be false

35   positives. Alternatively, they may be indicators of

additional independent loci. Our interpretation of these peaks can be enhanced by comparison to previous linkage reports of OA. Wright et al[12] have reported a linkage analysis of 12 markers on chromosome 2q in 66 nodal OA sib-pairs. Their 12 markers encompass over 65cM of 2q and in their study three were linked at $p \leq 0.05$: GCG, D2S326 and D2S126. These three markers flank D2S202, the marker that has the lowest p-value in our families: GCG and D2S326 are at least 20cM proximal to D2S202 whilst D2S126 is approximately 25cM distal to D2S202. Five of the 12 Wright et al markers are located in the interval in-between GCG/D2S326 and D2S126 and are therefore closer to D2S202. However, none of these supported linkage at $p \leq 0.05$. Of the 12 markers used by Wright et al D2S326 was the only one that was also used in our study. In our un-stratified families this marker had a LOD score of 0.45 (p=0.07) (Table 3). The majority of the families used by Wright et al contained female-only affected sib pairs. When we stratified for sex, the LOD score at D2S326 increased in our female-only pairs to 0.94 (p=0.019) but decreased in our male-only pairs to 0.18 (p=0.18) (Table 4). In our female/hips the LOD score was 0.74 (p=0.032). This female-specific linkage may therefore account for the peak observed in our un-stratified families that exists in-between markers D2S2330 and D2S326 (Figure 1). Intriguingly, this peak is also present when we stratify for hips-only (Figure 3) but is much less pronounced when we stratify for males-only (Figure 2) or male/hips (Figure 4). Our results for D2S326 point to a predominantly female-specific locus that is proximal to the predominantly male-specific locus that we have detected close to D2S202/D2S72.

Leppavuon et al[13] have also reported linkage to chromosome 2q in 42 affected sib pairs who have distal interphalangeal (DIP) OA, with a maximum LOD score of 4.06. They do not report the markers that they used in their study but instead give a cytogenetic location of 2q12-q14.2. This

18

is at least 70cM proximal to our major linkage at
D2S202/D2S72, which cytogenetically map to 2q31. One of our
chromosome 2 markers does map to 2q12-q14.2: D2S160. In our
un-stratified families this marker had a LOD score of 0.65

5   (p=0.041) (Table 3). In our un-stratified data there is
moderate evidence of linkage in-between markers D2S160 and
D2S142, with a MMLS of 0.95 (Figure 1). This linkage extends
from 2q14.1-2qq. Leppavuori et al did not report the sex-
structure of their families. When we stratified our families

10   for female-only and male-only affected pairs, neither group
supported linkage at D2S160 (p=0.32 for female-only pairs and
p=0.35 for male-only pairs) or at D2S114 (p=0.29 for female-
only pairs and p=0.11 for male-only pairs). However, in our
hip-only pairs there was evidence of linkage at D2S160

15   (p=0.028) and D2S114 (p=0.022). Overall, it may be that our
results do represent a confirmation of the linkage result of
Leppavuori et al but that this locus confers only a moderate
degree of susceptibility in our families. When we analyse
our data as a whole (481 families) we have the power to

20   detect linkage, as we do when we stratify for hip-only
families (311 families). However, when we stratify for
female-only (196 families) or male-only (102 families) we no
longer have the power to detect linkage.

In conclusion our linkage analysis of chromosome 2,
25   together with results previously reported, suggest that there
may be up to three distinct OA susceptibility loci on 2q, one
or more of which may demonstrate differential penetrance
between males and females: a locus close to D2S114 that may
be a susceptibility locus for females and males, a locus in-
30   between D2S2330 and D2S326 that may be predominantly be a
susceptibility locus for females and a locus close to or in-
between D2S117 and D2S325 that may predominantly be a
susceptibility locus for males.

35   Markers from other chromosomes

19

Since stratification analysis of chromosome 2 revealed
apparent significant differences between the different
categories examined, we also stratified 12 other markers that
had a p≤0.05 in stage 1 and which were not on chromosome 2.
5   The majority of these 12 markers will of course represent
false positives from our stage 1 analysis: the p-value for
each increased when the combined analysis was compared to
stage 1 (Table 2). Nevertheless, they cannot all be
discounted. Table 5 lists the stratification results for
10   these 12 markers.

Two of the 12 markers are of interest when one considers the
results of previous studies: D6S273 and DXS1068.

15   D6S273 maps close to the HLA complex on chromosome 6p and
Pattrick et al[14] have reported association of nodal OA with a
specific allele of the HLA-A gene. Furthermore, the COL11A2
gene, which encodes the α2 chain of the cartilage collagen
type XI, is tightly linked to the HLA complex. This gene has
20   been linked to and found to harbour causal mutations for the
osteochondrodysplasia Stickler syndrome[15]. This syndrome has
severe, precocious OA as one of its many phenotypic
components.

25   Leppavuori et al[13] have reported linkage of DIP OA to
chromosome 2q12-q14.2. A second linkage that they also
reported maps to cytogenetic band Xp11.3. DXS1068 also maps
to Xp11.3.

30   Association Analysis
Having established evidence for linkage to chromosome 2, the
chromosome 2 markers were then tested for association. We
used the Transmit software program[16] to test for association
with linkage disequilibrium by the transmission
35   disequilibrium test.

Since there are three possible distinct susceptibility loci on chromosome 2q we tested for disequilibrium for all 13 of our 2q markers. We tested the genotyping data without stratification and with the following stratification criteria: female-only pairs, male-only pairs, hip-only pairs, female/hip pairs and male/hip pairs.

Only D2S2330 demonstrated disequilibrium in the un-stratified data set, with a global $\chi^2_{5df} \equiv 11.43$ (0.05>p>0.02) (Table 6). This was accounted for by the significant distorted transmission of two alleles (0.05>p>0.02), one of which demonstrated excess transmission whilst the second demonstrated reduced transmission. In the stratified data disequilibrium was also positive for D2S2330 in the male/hips with global $\chi^2_{4df} \equiv 12.14$ (0.02>p>0.01). However, no one allele demonstrated significant distorted transmission and the allele that demonstrated excess transmission in the un-stratified data demonstrated reduced transmission in the male/hips. It is likely therefore that the positive disequilibrium results for D2S2330 are false positives.

Disequilibrium was also positive in the stratified data for two additional markers: D2S325 and D2S117. D2S325 demonstrated positive disequilibrium in male-only pairs with global $\chi^2_{2df} \equiv 6.89$ (0.05>p>0.02). This disequilibrium was accounted for by the significant (0.05>p>0.02) distorted transmission of two alleles (200bp allele [allele 1] as listed in the GDB and 190bp allele [allele 4] as listed in the GDB), one of which (the 190 bp allele) demonstrated excess transmission. D2S117 demonstrated positive disequilibrium in female-only pairs with global $\chi^2_{3df}=8.05$ (0.05>p>0.02) and in male/hip pairs with global $\chi^2_{4df}= 12.89$ (0.02>p>0.01). The positive disequilibrium in the female-only pairs was accounted for by the significant distorted transmission of three alleles, two of which demonstrated excess transmission (0.05>p>0.01) (192 bp allele [allele 3] as listed in the GDB and 202 bp allele [allele 6] as listed

in the GDB) whilst the third demonstrated reduced
transmission (0.05>p>0.02) (208bp allele [allele 9] as listed
in the GDB). The positive disequilibrium in the male/hips
was accounted for by a single allele (208bp allele [allele 9]
as listed in the GDB): observed transmission in male/hips of
85 compared to expected transmission of 74.63 ($\chi^2_{1df} \equiv 8.15$,
0.01>p>0.001). Although our male-only pairs did not
demonstrate significant disequilibrium at D2S117 the
transmission of alleles did approach significance in this
category with a global $\chi^2_{3df} \equiv 7.73$ (0.1>p>0.05). In these
male-only pairs allele 208bp did demonstrate distorted
transmission with an observed transmission of 128 compared to
an expected transmission of 117.20 ($\chi^2_{1df} \equiv 5.95$, 0.02>p>0.01).
Intriguingly, it was the 208 bp allele that demonstrated
reduced transmission in our female-only pairs with an
observed transmission of 206 compared to an expected
transmission of 218.4($\chi^2_{1df} \equiv 4.26$, 0.05>p>0.02).

Methods

Affected sibling-pairs

Families were recruited which contained at least two siblings
two had undergone one or more total hip (THR) and/or total
knee replacements (TKR) for idiopathic OA. Clinically these
patients are at the severe end of the OA spectrum with
advanced radiological changes. The idiopathic diagnosis was
supported by clinical, radiological, operative and
histological findings: patients who had undergone joint
replacement surgery secondary to other factors, such as
intra-articular fracture or rheumatoid arthritis, were
excluded. Families were ascertained at three centres within
the United Kingdom: The Nuffield Orthopaedic Centre in
Oxford, the Royal Orthopaedic Hospital in Birmingham and
Musgrave Park Hospital in Belfast. Idiopathic OA is
typically a late-onset disease and parents of affected
siblings are rarely available. Of the 481 families used in

the study none contained a parent who was able to
participate. We therefore collected siblings who had not
undergone joint replacement to assist in the determination of
identity-by-descent (IBD) allele transmittance. The 481

5   families were comprised of 1052 affected individuals plus an
additional 302 unaffected siblings (Table 1). 59.3% of the
affected individuals were female, 40.7% were male. For each
individual, 25ml of venous blood was collected into EDTA
tubes and DNA was extracted by conventional techniques.

10

Markers and Genotyping

Our initial screening panel of 272 microsatellite markers was
essentially the panel used by Reed et al[8]. The additional
microsatellite markers used to provide a denser coverage of

15   chromosome 11 were obtained from the GDB or from the ABI
Prism Linkage Mapping Set (Version 2). The markers were
amplified using conventional conditions with either the
forward or the reverse primer in a PCR pair fluorescently
labelled. The amplification products were electrophoresed

20   through 6% acrylamide using an Applied Biosystems 377
Automated DNA Sequencer[B]. Alleles were sized using Applied
Biosystems Genescan version 2.0.2. and Genotyper version 1.1
software.

25   Linkage and Linkage disequilibrium analysis
Initially error checking procedures were employed for all
families for each marker. After identification of
straightforward mis-inheritances, more subtle transmission
errors were detected using the PedCheck program[17]. The entire

30   family data set was tested with Relative which is able to
produce a probability calculation (based on 50 or more
unlinked markers) that full sibships are in fact half sibs or
even unrelated (due to unknown adoption of laboratory error).
All 481 families used in the study successfully progressed

35   through these checks. In addition markers were checked for

having excess homozygotes, based on their allele frequencies
and heterozygosities. Markers shown to be unreliable were
eliminated from the study. Finally the marker data were
haplotyped for each chromosome using Simwalk2. This checks

5   for areas on the chromosome where excessive recombination
events may alert us to genotyping errors or mis-assignment of
a marker position.

Non parametric linkage analysis was performed using the

10   SIBPAIR module of the ANALYZE package[18]. This module is able
to use data from siblings to determine identity-by-descent
(IBD) allele transmittance. In this way it is appropriate to
our study since we were unable to collect parents of our
affected siblings. In the linkage analysis, siblings who had

15   not undergone joint replacement were given a clinical status
of unknown. The SIBPAIR module produces a singlepoint LOD
score and its asymptotic P-value. Allele frequencies were
calculated from the input data using either GAS or Downfreq
(part of the ANALYZE package). Subsequent multipoint

20   analyses was performed using ASPEX which calculates its own
allele frequencies from the data set, using a maximum
likelihood method, and employs marker information across the
chromosome simultanenously[19]. ASPEX produces maximum LOD
score (MLS) under an additive model. In addition it produces

25   an exclusion map along the entire chromosome based on a fixed
value for λs.

We tested for linkage disequilibrium by the transmission
disequilibrium test (TDT) using the TRANSMIT software

30   program[16]. Alleles with a frequency <0.1 were pooled. A
global χ^2 statistic was computed for each microsatellite. If
a microsatellite was significant a χ^2 statistic was then
computed for its individual alleles.

35   Stratification

24

We stratified for sex, for joint replaced (hip or knee) and for sex combined with joint replaced.

5   For those families with more than two affected siblings and in which the siblings were not of the same sex, the affected sibling of opposite sex to a same pair was given an affected status of unknown in the linkage analysis. In this way we were stratifying for sex whilst not excluding siblings who could be used to assist in the determination of identity-by-
10   descent (IBD) allele transmittance.

A hip-only pair were siblings who had each undergone joint replacement of the hip only (mono or bi-lateral) whilst a knee-only pair had undergone joint replacement of the knee
15   only (mono or bi-lateral). If an affected pair was composed of one sibling who had undergone joint replacement of one joint type only (hip or knee) whilst their affected sibling had undergone joint replacement of the hip and knee then that pair were excluded. For an affected trio, if a pair of the
20   siblings had both undergone joint replacement of the same joint type only (hip or knee) whilst the third sibling had undergone both hip and knee replacement, then the concordant pair were used in the stratification study whilst the third sibling was given an unaffected status in the linkage
25   analysis. Again, we were attempting to maximise our determination of IBD allele transmittance.

Table 1(a)

Table 1   a) The families used in stages 1 and 2 together with the combined total figure. Also listed are the pairs concordant for different stratification criteria. b) A list of the individuals in the study.

a)

Families

	Stage 1	Stage 2	Total
Families	297	184	481
sibling pair	265	150	415
sibling trio	23	27	50
sibling quattro	7	5	12
other[a]	2	2	4
Female only	132	64	196
pair	121	59	180
trio	11	5	16
Male only	61	41	102
pair	57	36	93
trio	3	5	8
other[a]	1	0	1
Hip only	194	117	311
pair	170	96	266
trio	18	17	35
quattro	4	2	6
other[a]	2	2	4
Knee only	34	20	54
pair	33	19	52
trio	1	1	2
Female/hip only	85	47	132
pair	77	46	123
trio	8	1	9
Female/knee only	16	5	21
pair	16	5	21
Male/hip only	45	27	72
pair	41	25	66
trio	3	2	5
other[a]	1	0	1
Male/knee only	4	4	8
pair	4	4	8

Table 1(b)

b)

Individuals

	Stage 1	Stage 2	Total
Affected individuals	641	411	1052
Female	394	230	624
Male	247	181	428
Hip only	479	309	788
Knee only	121	77	198
Hip & Knee	41	25	66
Female/hip only	287	172	459
Female/knee only	77	42	119
Female/hip & knee	30	16	46
Males/hip only	192	137	329
Males/knee only	44	35	79
Males/hip & knee	11	9	20
Additional siblings[b]	211	91	302
Female	107	49	156
Male	104	42	146

[a]Other relative pairs such as cousins, uncles, aunts.
[b]Since our families lack parents, siblings who had not undergone joint replacement surgery were collected to assist in the determination of parental genotypes. These siblings were given an "unknown" clinical status in the linkage analysis.

Table 2 LOD scores and p-values for all markers that had a p≤0.05 in screen 1, for these markers in screen 2 and for screens 1 and 2 combined (* = p≤0.05).

| Marker | STAGE 1 | | STAGE 2 | | COMBINED | |
	p-value	LOD	p-value	LOD	p-value	LOD
D2S202	0.036*	0.70	0.07	0.49	0.009*	1.21
D3S1266	0.017*	0.96	0.5	0.00	0.082	0.42
D4S231	0.040*	0.67	0.5	0.00	0.33	0.04
D4S415	0.018*	0.95	0.33	0.04	0.025*	0.83
D6S260	0.050*	0.58	0.5	0.00	0.13	0.29
D6S273	0.016*	0.98	0.5	0.00	0.077	0.44
D6S286	0.030*	0.77	0.5	0.00	0.081	0.42
D6S281	0.046*	0.61	0.45	0.00	0.062	0.52
D7S669	0.018*	0.94	0.25	0.10	0.021*	0.90
D7S530	0.006*	1.33	0.41	0.01	0.013*	1.09
D11S907	0.050*	0.58	0.12	0.31	0.019*	0.92
D11S903	0.017*	0.97	0.07	0.49	0.004*	1.45
D11S901	0.0004*	2.45	0.10	0.37	0.0003*	2.51
D17S807	0.014*	1.03	0.5	0.00	0.15	0.24
D17S789	0.010*	1.16	0.5	0.00	0.071	0.47
DXS1068	0.020*	0.84	0.5	0.00	0.10	0.35

Table 3   LOD scores and p-value for stages 1 and 2 combined for the chromosome 2 markers.

Marker	cM from 2p telomere	p-value	LOD
D2S139	122	0.50	0.00
D2S160	145	0.041*	0.65
D2S114	167	0.050*	0.58
D2S142	186	0.28	0.07
D2S2330	194	0.19	0.16
D2S326	203	0.07	0.45
D2S364	212	0.08	0.41
D2S117	221	0.21	0.14
D2S202	223	0.009*	1.21
D2S72	225	0.007*	1.26
D2S325	231	0.09	0.39
D2S2382	242	0.31	0.05
D2S159	259	0.5	0.00

Table 4 Stratification of stages 1 and 2 combined of the chromosome 2 markers for affected female-only pairs, affected male-only pairs, hip-only pairs, knee-only pairs, affected females with hip-only disease and affected males with hip-only disease.

Marker	cM	FEMALES		MALES		HIPS		KNEES		FEMALE/HIPS		MALE/HIPS	
		p-value	LOD	p-value	LOD	p-value	LOD	p-value	LOD	p-value	LOD	p-value	LOD
D2S139	122	0.50	0.00	0.50	0.00	0.50	0.00	0.50	0.00	0.50	0.00	0.50	0.00
D2S160	145	0.32	0.04	0.35	0.03	0.028*	0.79	0.29	0.07	0.080	0.42	0.50	0.00
D2S114	167	0.29	0.06	0.11	0.32	0.022*	0.88	0.50	0.00	0.11	0.33	0.30	0.06
D2S142	186	0.50	0.00	0.052	0.57	0.099	0.36	0.50	0.00	0.50	0.00	0.008*	1.28
D2S2330	194	0.28	0.07	0.14	0.25	0.12	0.31	0.50	0.00	0.36	0.03	0.066	0.49
D2S326	203	0.019*	0.94	0.18	0.18	0.093	0.38	0.50	0.00	0.032*	0.74	0.13	0.28
D2S364	212	0.38	0.02	0.054	0.56	0.067	0.49	0.070	0.47	0.20	0.15	0.043*	0.64
D2S117	221	0.43	0.01	0.18	0.19	0.047*	0.61	0.14	0.25	0.19	0.16	0.037*	0.70
D2S202	223	0.40	0.01	0.0021*	1.79	0.0005*	2.36	0.34	0.03	0.20	0.15	0.0016*	1.89
D2S72	225	0.22	0.13	0.0025*	1.70	0.017*	0.97	0.22	0.13	0.11	0.34	0.0066*	1.34
D2S325	231	0.09	0.39	0.46	0.00	0.017*	0.98	0.50	0.00	0.13	0.28	0.30	0.06
D2S2382	242	0.49	0.00	0.47	0.00	0.26	0.09	0.17	0.20	0.16	0.22	0.50	0.00
D2S159	259	0.50	0.00	0.43	0.01	0.50	0.00	0.50	0.00	0.25	0.10	0.36	0.03

Table 5   Stratification of stages 1 and 2 combined of the 12 markers that had a p≤0.05 for stage 1 (excluding the chromosome 2 and 11 markers) for affected female-only pairs, affected male-only pairs, hip-only pairs, knee-only pairs, affected females with hip-only disease and affected males with hip-only disease.

Marker	All Families		Females		Males		Hips		Knees		Female/Hips		Male/Hips	
	p	LS	p	LS	p	LS	p	LS	p	LS	p	LS	p	LS
D3S1266	0.082	0.42	0.0060*	1.37	0.25	0.09	0.29	0.07	0.0035*	1.58	0.0067*	1.33	0.42	0.01
D4S231	0.33	0.04	0.045*	0.62	0.16	0.21	0.066	0.49	0.10	0.35	0.0052*	1.42	0.26	0.09
D4S415	0.025*	0.83	0.15	0.22	0.11	0.34	0.20	0.16	0.12	0.30	0.50	0.00	0.24	0.11
D6S260	0.13	0.29	0.16	0.22	0.50	0.00	0.19	0.17	0.50	0.00	0.50	0.00	0.33	0.04
D6S273	0.077	0.44	0.0034*	1.59	0.50	0.00	0.18	0.18	0.044*	0.63	0.0053*	1.42	0.5	0.00
D6S286	0.081	0.42	0.13	0.27	0.35	0.03	0.016*	0.99	0.50	0.00	0.058	0.54	0.36	0.03
D6S281	0.062	0.52	0.37	0.03	0.50	0.00	0.11	0.33	0.40	0.01	0.17	0.20	0.50	0.00
D7S669	0.021*	0.90	0.071	0.47	0.081	0.42	0.010*	1.16	0.39	0.02	0.032*	0.74	0.16	0.22
D7S530	0.013*	1.09	0.090	0.39	0.15	0.24	0.034*	0.72	0.17	0.19	0.18	0.18	0.29	0.07
D17S807	0.15	0.24	0.32	0.05	0.5	0.00	0.12	0.30	0.50	0.00	0.18	0.18	0.25	0.10
D17S789	0.071	0.47	0.12	0.29	0.39	0.02	0.25	0.10	0.021*	0.90	0.28	0.07	0.20	0.16
DXS1068	0.10	0.35	0.20	0.16	0.049*	0.60	0.19	0.17	0.26	0.09	0.50	0.00	0.02*	0.90

Table 6
TRANSMIT of chromosome 2.
χ2 analysis
(figures in brackets = degrees of freedom)

Locus	All data	Females-only	Males-only	Hips-only	Female/Hips	Male/Hips
D2S139	(5) 3.74	(5) 6.60	(5) 3.26	(5) 2.62	(5) 4.70	(5) 4.83
D2S160	(3) 3.19	(4) 1.80	(3) 3.51	(3) 5.69	(4) 5.96	(4) 3.32
D2S114	(4) 1.83	(5) 2.74	(4) 4.36	(4) 2.88	(5) 4.03	(4) 3.77
D2S142	(4) 2.52	(4) 2.18	(4) 3.52	(4) 0.87	(4) 1.25	(4) 2.44
D2S2330	(5) 11.43*	(5) 7.90	(5) 6.67	(5) 10.91	(5) 8.12	(4) 12.14*
D2S326	(4) 5.04	(5) 6.78	(4) 2.98	(4) 2.80	(4) 1.11	(4) 7.07
D2S364	(4) 5.59	(4) 3.89	(4) 3.43	(4) 4.37	(4) 1.28	(4) 4.42
D2S117	(3) 5.38	(3) 8.05*	(3) 7.73	(3) 5.31	(3) 5.19	(4) 12.89*
D2S202	(3) 3.59	(3) 3.98	(3) 0.50	(3) 3.01	(3) 6.32	(3) 1.45
D2S72	(3) 5.02	(4) 5.34	(3) 0.56	(3) 2.82	(4) 5.19	(3) 2.04
D2S325	(2) 3.51	(2) 1.00	(2) 6.89*	(2) 3.12	(3) 1.30	(3) 5.61
D2S2382	(2) 2.30	(2) 3.72	(2) 2.42	(2) 1.26	(2) 3.42	(2) 1.80
D2S159	(4) 1.40	(4) 3.92	(4) 1.94	(4) 0.90	(4) 4.49	(4) 1.15

32

References

1.   Spector T.D., Cicuttini F., Baker J., Loughlin J. & Hart D. Genetic influences on osteoarthritis in women: a twin study. Br. Med. J. 312, 940-943 (1996).

2.   Chitnavis J., Sinsheimer J.S., Clipsham K., Loughlin J., Sykes B., Burge P.D. & Carr A.J. Genetic influences in end-stage osteoarthritis. Sibling risks of hip and knee replacement for idiopathic osteoarthritis, J. Bone. Joint. Surge 79, 660-664   (1997).

3.   Felson D.T., Couropmitree N.N., Chaisson C.E., Hannan M.T., Zhang Y., McAlindon T.E., LaValley M., Levy D. & Myers R.H. Evidence for a Mendelian gene in a segregation analysis of generalized radiographic osteoarthritis. Arth. Rheum. 41, 1064-1071 (1998).

4.   Hirsch R., Lethbridge-Cejku M., Hanson R., Scott W.W., Reichle R., Plato C.C., Tobin J.D. & Hochberg M.C. Familial aggregation of osteoarthritis. Arth. Rheum. 41, 1227-1232 (1998).

5.   Creamer P. & Hochberg M.C. Osteoarthritis. Lancet 350, 503-508 (1997).

6.   Jones A. & Doherty M. ABC of Rheumatology. Osteoarthritis. Br. Med. J. 310,   457-460 (1995).

7.   Horton W.A. Evolution of the bone dysplasia family. Am. J. Med. Genet. 63, 4-6   (1996).

8.   Reed P.W., Davies J.L., Copeman J.B., et al. Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nature Genet. 7,   390-395 (1994).

9.   Kaprio J., Kujala U.M., Peltonen L. & Koskenvuo M.
Genetic liability to osteoarthritis may be greater in women
than men. Br. Med. J. 313, 232, (1996).

10.   Lindberg H. Prevalence of primary coxarthrosis in
siblings of patients with primary coxarthrosis. Clin.
Orthop. 203, 273-275 (1986).

11.   Cooper C., McAlindon T., Snow S., et al. Mechanical and
constitutional risk factors for symptomatic knee
osteoarthritis: differences between medial tibio-femoral and
patello-femoral disease. J. Rheumatol. 21, 307-313 (1994).

12.   Wright G.D., Hughes A.E., Regan M. & Doherty M.
Association of two loci on   chromosome 2q with nodal
osteoarthritis. Ann. Rheum. Dis. 55, 317-319
(1996).

13.   Leppavuori J.K., Kujala U.M., Kaprio J., Nissila M.,
Helliovaara M., Kinnunen J., Koskenvuo M. & Peltonen L.
Genome screen for predisposing loci of distal
interphalangeal joint osteoarthritis. Am. J. Hum. Genet.
Suppl. 63, A1715 (1998).

14.   Pattrick M., Manhire A., Ward A.M. & Doherty M. HLA-A, B
antigens and αI-antitrypsin phenotypes in nodal generalised
osteoarthritis and erosive osteoarthritis.   Ann. Rheum. Dis.
48, 470-475 (1989).

15.   Mundlos S. & Olsen B.R. Heritable diseases of the
skeleton. Part II: Molecular insights into skeletal
development-matrix components and their homeostasis.
FASEB J. 11, 227-233 (1977).

16.   Clayton D. Transmit, http://www.mrc-

bsu.cam.ac.uk/pub/methodology/genetics/transmit (1997).

17.   O'Connell J.R. & Weeks D.E. PedCheck: a program for
identifying marker typing incompatibilities in linkage
analysis. Am. J. Hum. Genet. Suppl. 60, A288 (1997).

18.   Terwilliger J. Program SIBPAIR: sibpair analysis on
nuclear families.
1996;ftp://linkage.cpmc.columbia.edu.

19.   Hinds D. & Risch N. The ASPEX package: affected sibpair
mapping.
1996;ftp://lahmed.stanford.edu/pub/aspex.

35

Claims

1.   A method for identifying individuals susceptible to
osteoarthritis comprising obtaining a sample of genomic DNA
and detecting the presence or absence of any one of the 190
bp and the 200 bp allele of D2S325 from chromosome 2.

2.   A method according to claim 1 for wherein the
individuals are male.

3.   A method for identifying individuals susceptible to
osteoarthritis comprising obtaining a sample of genomic DNA
and detecting the presence or absence of any one of the 192
bp, 202bp and 208 bp allele of D2S117 from chromosome 2.

4.   A method according to claim 3 for identifying male
individuals susceptible to osteoarthritis comprising
obtaining a sample of genomic DNA and detecting the presence
or absence of the 208 bp allele of D2S117 from chromosome 2.

5.   A method according to claim 4 for identifying male
individuals susceptible to osteoarthritis of the hip.

6.   A method for isolating genetic loci associated with
susceptability to OA comprising screening genomic libraries
with sequence from the 190 bp allele of D2S325 and
identifying open reading frames in regions adjacent to said
allele.

7.   A method for isolating genetic loci associated with
susceptability to OA comprising screening a genomic library
from an individual who is homozygote for the 190 bp allele of
D2S325 and identifying open reading frames in regions
adjacent to said allele.

8.   A method for isolating genetic loci associated with
susceptability to OA comprising identifying open reading
frames in regions adjacent to D2S325 and comparing said open
reading frames in individuals carrying a 190 bp allele of
D2S325 with said open reading frames in individuals with a
non-190 bp allele of D2S325.

9.   A method for isolating genetic loci associated with
susceptability to OA comprising screening genomic libraries
with sequence from the 192 bp and 202 bp alleles of D2S117
and identifying open reading frames in regions adjacent to
said allele.

10.   A method for isolating genetic loci associated with
susceptability to OA comprising screening a genomic library
from an individual who is homozygote for any one of the 192
bp, the 202 bp and the 208 bp alleles of D2S117 and
identifying open reading frames in regions adjacent to said
allele.

11.   A method for isolating genetic loci associated with
susceptability to OA comprising identifying open reading
frames in regions adjacent to D2S117 and comparing said open
reading frames in individuals carrying any one of the 192 bp,
202bp and 208bp alleles of D2S117 with said open reading
frames in individuals with other alleles of D2S117.

12.   A method for isolating genetic loci according to any one
of claims 7 to 11 in which open reading frames are identified
within 500 Kb of said allele.

13.   The use of the 190 bp allele of D2S325 as a marker for
the identification of loci influencing susceptibility to OA.

14.   The use of the 192 bp, 208bp and 202 bp alleles of

37

D2S117 as markers for the identification of loci influencing
susceptibility to OA.

15.   A method for mapping loci which affect susceptibility to
OA by comparing genomic regions containing the 208 bp allele
of D2S117 with genomic regions containing other alleles of
D2S117.

16.   A method for determining individual susceptibility to
osteoarthritis comprising obtaining sample genomic DNA from
siblings, at least two of which have clinical symptoms of
osteoarthritis, analysing a region of their genomic DNA
comprising a polymorphic marker, said region being located on
chromosome 2q between D2S117 and D2S325, identifying allele
sharing between the siblings as defined by a maximum log of
the odds (LOD) score of greater than 1 and a p-value of less
then 0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

17.   A method for determining individual susceptibility to
osteoarthritis comprising obtaining sample genomic DNA from
siblings, at least two of which have clinical symptoms of
osteoarthritis, analysing a region of their genomic DNA
comprising the polymorphic marker D2S114, identifying allele
sharing between the siblings as defined by a maximum log of
the odds (LOD) score of greater than 1 and a p-value of less
then 0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

18.   A method for determining individual susceptibility to
osteoarthritis comprising obtaining sample genomic DNA from
siblings, at least two of which have clinical symptoms of
osteoarthritis, analysing a region of their genomic DNA
comprising a polymorphic marker, said region being located on
chromosome 2q between D2S330 and D2S326, identifying allele

sharing between the siblings as defined by a maximum log of
the odds (LOD) score of greater than 1 and a p-value of less
then 0.25, and determining individual susceptibility to
osteoarthritis by reference to the allele sharing.

19. A method for determining individual susceptibility to
osteoarthritis according to any one of claims 13 to 15 in
which one or more of the following genomic regions is
additionally analysed; a genomic region comprising the
polymorphic marker D6S273 and a genomic region comprising the
polymorphic marker DXS1068.

20. A method for determining individual susceptibility to
osteoarthritis comprising obtaining sample genomic DNA from
siblings, at least two of which have clinical symptoms of
osteoarthritis, analysing a region of their genomic DNA
comprising any one of the polymorphic markers; D2S202,
D3S1266, D4S231, D4S415, D6S260, D6S273, D6S286, D6S281,
D7S669, D7S530, D11S907, D11S903, D11S901, D17S807, D17S789,
DXS1068, identifying allele sharing between the siblings as
defined by a maximum log of the odds (LOD) score of greater
than 1 and a p-value of less then 0.25, and determining
individual susceptibility to osteoarthritis by reference to
the allele sharing.

21. A method for identifying loci conferring susceptibilty
to osteoarthritis comprising screening a genomic library with
genetic sequence derived from one or more of the following
polymorphic markers; D2S202, D3S1266, D4S231, D4S415, D6S260,
D6S273, D6S286, D6S281, D7S669, D7S530, D11S907, D11S903,
D11S901, D17S807, D17S789, DXS1068 and identifying open
reading frames in regions adjacent to the marker.

22. A method according to claim 18 in which the open reading
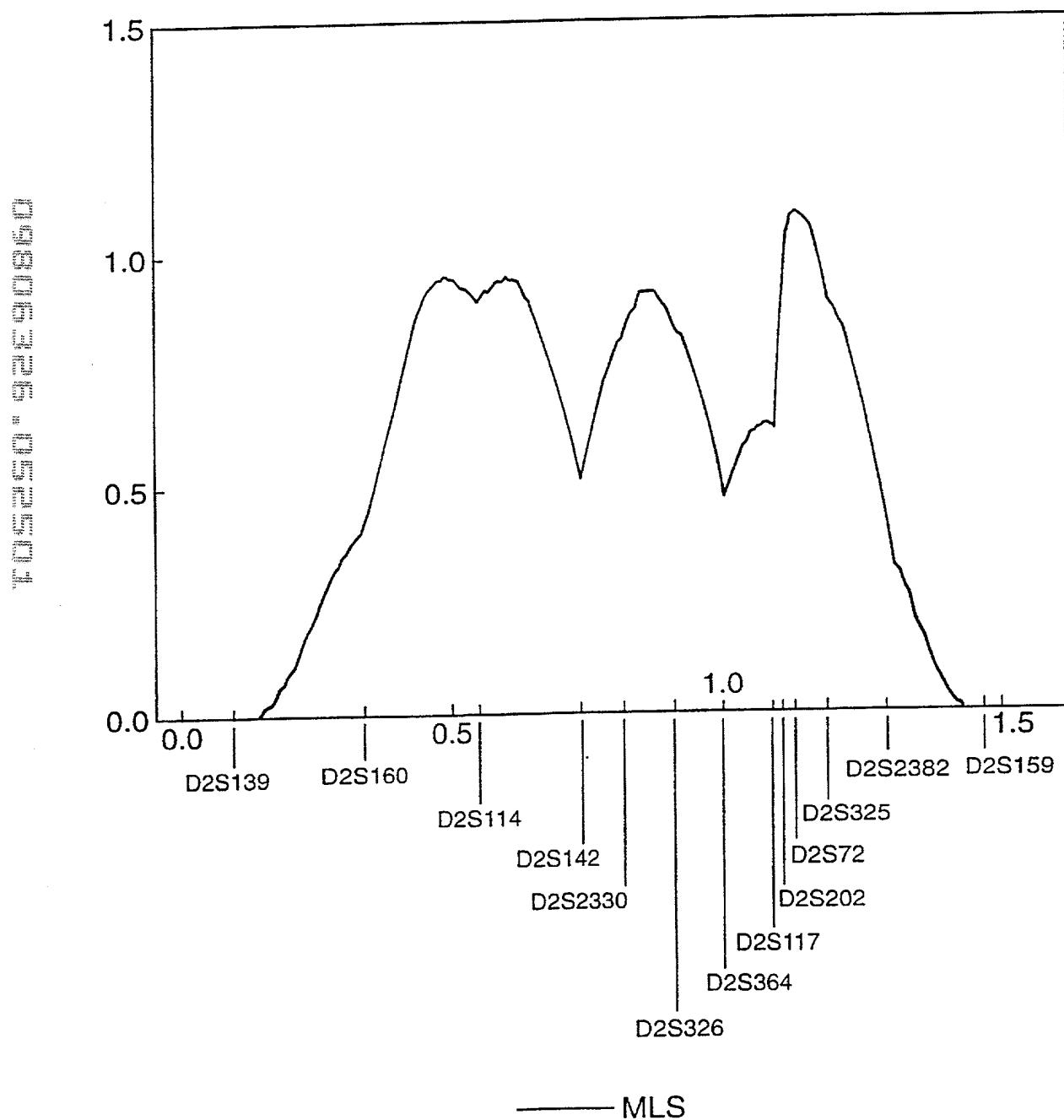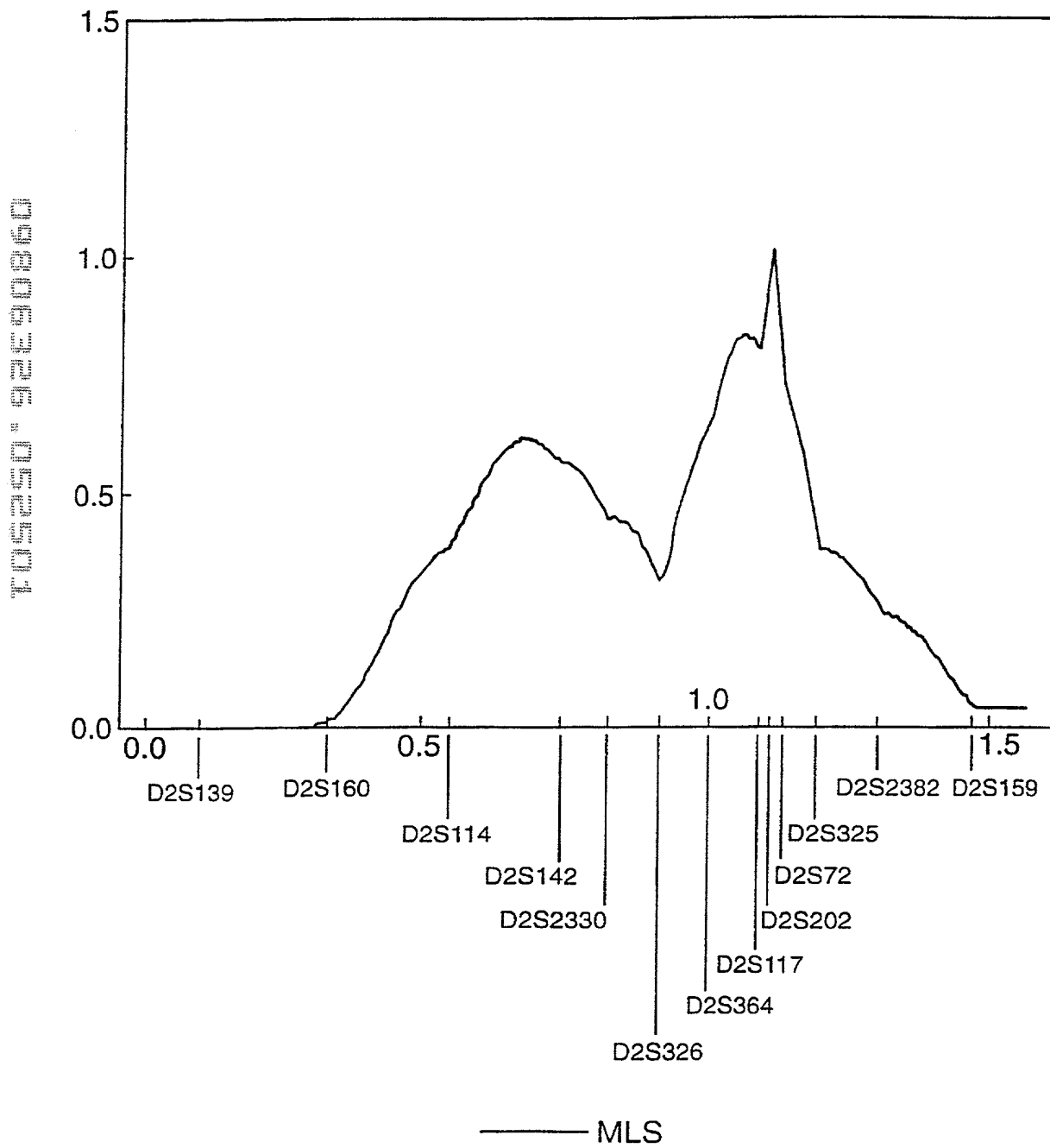frames identified are located within 500 Kb of the

polymorphic marker.
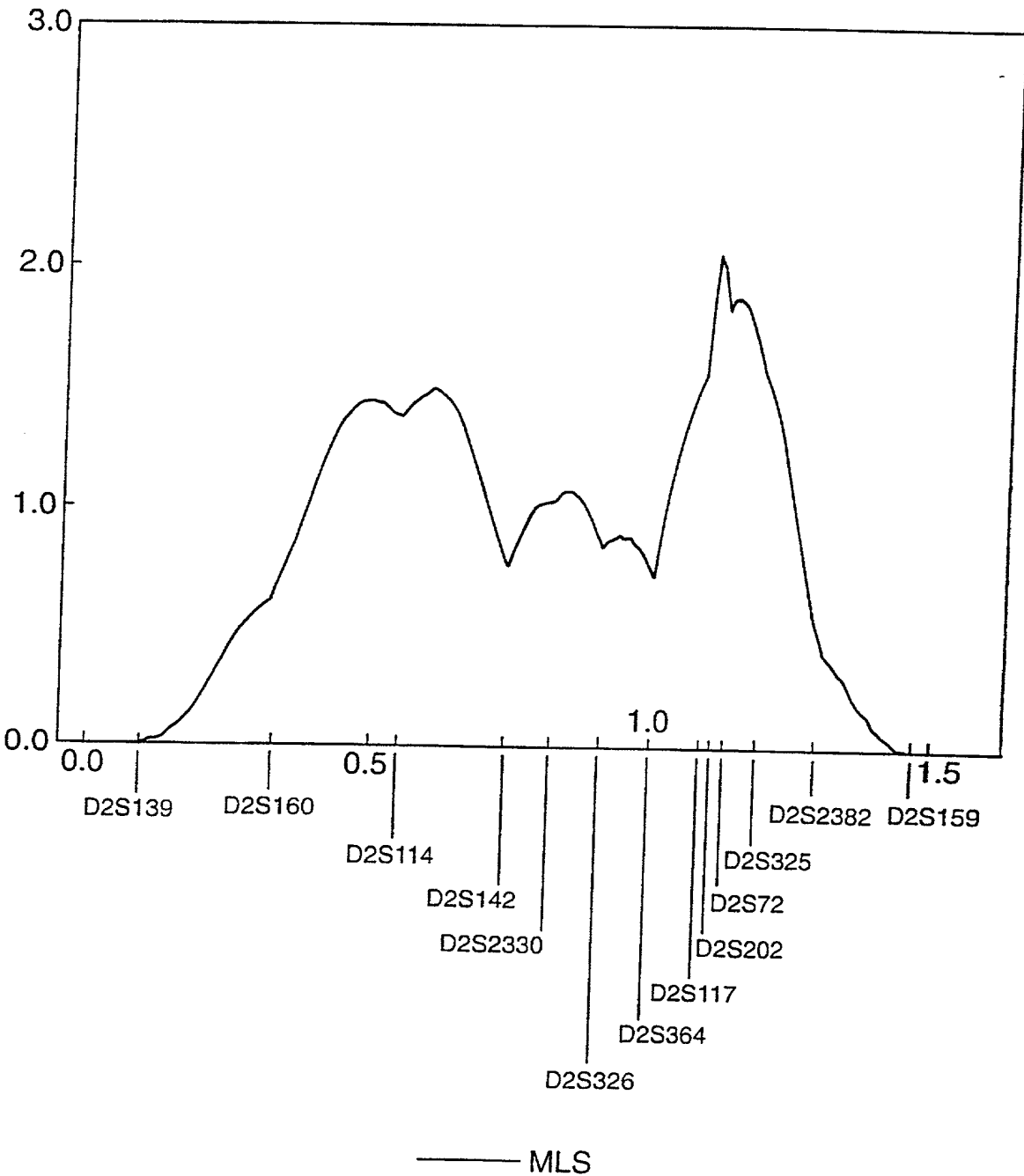
FIG. 1
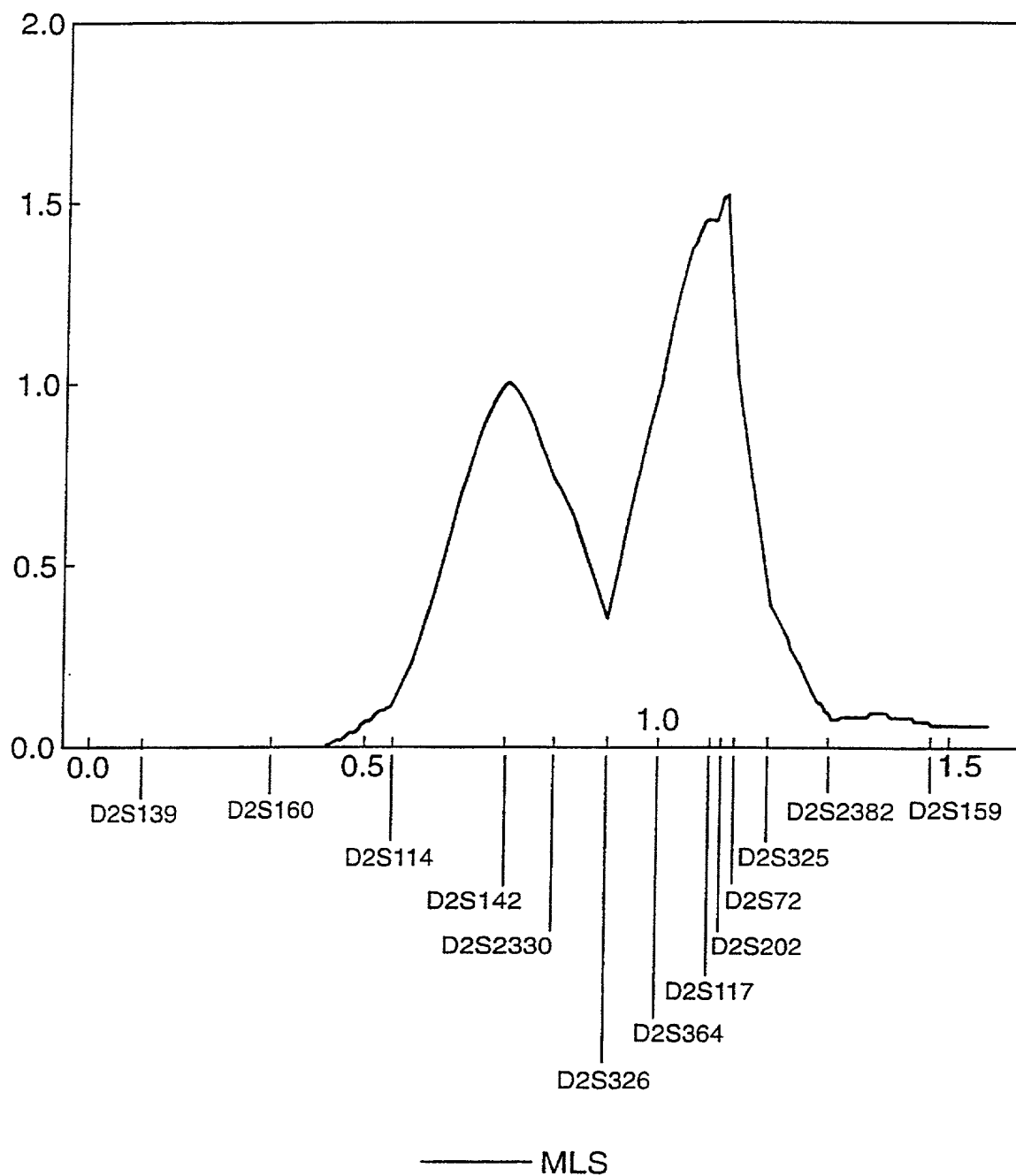

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FIG. 2

FIG. 3

— MLS

4/4

FIG. 4

————— MLS

RULE 63 (37 C.F.R. 1.63)
INVENTORS DECLARATION FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, mailing address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

SUSCEPTIBILITY LOCUS FOR OSTEOARTHRITIS

the specification of which (check applicable box(s)):

☐   is attached hereto
☐   was filed on _____ as U.S. Application Serial No. _____ (Atty Dkt. No. 620-136)
☒   was filed as PCT International application No. _____ PCT/GB99/03264   on   4 October 1999
and (if applicable to U.S. or PCT application) was amended on _____

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose to the Patent Office all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed before the filing date of this application:

Priority Foreign Application(s):

Application Number	Country	Day/Month/Year Filed
9821427.3	GREAT BRITAIN	2 October 1998
9821428.1	GREAT BRITAIN	2 October 1998
9903441.5	GREAT BRITAIN	15 February 1999

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	Date/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below:

Prior U.S./PCT Application(s):   Status: patented
Application Serial No.   Day/Month/Year Filed   pending, abandoned
        PCT/GB99/03264   4 October 1999

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And on behalf of the owner(s) hereof, I hereby appoint NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8ᵗʰ Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed), and the following attorneys thereof (of the same address) individually and collectively owner's/owners' attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Larry S. Nixon, 25640; Arthur R. Crawford, 25327; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 39363; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr., 29366; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Presta, 19828; Joseph S. Presta, 35329; Joseph A. Rhoa, 37515; Raymond Y. Man, 41426; Chris Comuntzis, 31097. I also authorize Nixon & Vanderhye to delete any attorney names/numbers no longer with the firm and to act and rely solely on instructions directly communicated from the person, assignee, attorney, firm, or other organization sending instructions to Nixon & Vanderhye on behalf of the owner(s).

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